The Mechanism of Muscular Contraction

Recent structural studies suggest a revealing model for cross-bridge action at variable filament spacing.

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During the last few years the basic features of the sliding-filament model of contraction in striated muscle have gained general acceptance and it has been possible to concentrate attention on the detailed mechanism by which the relative sliding force between the actin and myosin filaments is developed. A number of observations have indicated in general outline how cross-bridges between the filaments may be involved in the generation of this force but have also revealed some apparently paradoxical properties of the system. The most recent findings show a possible way in which these paradoxes can be resolved. Furthermore, there is now a real possibility of solving the problem in complete detail, provided a way can be found to crystallize a recently purified globular subfragment of the myosin molecule. In this article I discuss these new findings and their implications.

According to the interdigitating filament model of striated muscle (*1*), the contractile material consists of long series of partially overlapping arrays of actin and myosin filaments (see Fig. 1) which form the myofibrils. These overlapping arrays give rise to the characteristic band pattern visible in the light microscope. In vertebrate striated muscle the myosin-containing filaments are spaced out in a hexagonal lattice 400 to 450 angstroms apart, with the actin-containing filaments in between them at the trigonal positions of the lattice. The space between the filaments is occupied by sarcoplasm (a dilute aqueous solution of salts and of other proteins).

When the muscle changes length, either actively during contraction or passively during stretch or during release from an extended length, the sliding filament model (*2*) supposes that the length of the filaments themselves remains essentially constant but that the overlapping arrays of filaments slide past each other, the actin being drawn further into the array of myosin filaments (which form the A-bands) as the muscle shortens, or withdrawn again as the muscle is stretched. The evidence for this model has been reviewed in a number of papers (*3–5*) and is mentioned only incidentally in this article.

While the overall changes in the arrangement of the filaments can be deduced from light-microscopic observations (once the origin of the band pattern is known), the underlying mechanism which produces movement of the filaments past each other is obviously not accessible to direct visual observation, and so we have to build up a picture of it in a less direct way, using whatever technique seems likely to give us useful clues.

Early Ideas about Cross-Bridges

The first and most crucial clue came from early electron-microscope observations of sections of muscle (*4*, *6*) which showed that cross-bridges linked the actin and myosin filaments together across the gap of about 130 angstroms which exists between their surfaces. The bridges could still be seen as projections on the myosin filaments in places where no actin filament lay alongside them (for example, on the outside of fibrils and in the H-zone of stretched sarcomeres), whereas they were not visible on the actin filaments in the I-bands. It was clear, therefore, that they formed a permanent part of the myosin filament structure. As they were the only visible mechanical agents by which a force could be developed between the actin and myosin filaments, it was suggested (*4*) that this indeed was their function, and that they very probably represented the heavy-meromyosin subunit of the myosin molecule. It was already known that the actin-combining ability and adenosine triphosphatase activity were associated with this part of the molecule (*7*), and it seemed reasonable to suppose that the sites responsible for these properties would be built into the overall structure of the muscle in such a way that they could interact directly with the actin filaments.

During the contraction of a muscle, even during a single twitch, the structure may shorten by 30 percent of its original length or more, and the actin and myosin filaments must therefore slide past each other (in a frog muscle starting at a resting sarcomere length of 2.5 microns) by 0.375 microns (that is, 3750 angstroms) in each half-sarcomere. Some variation in orientation of the cross-bridges can be seen in electron micrographs, but the distal ends never seem to be displaced by more than about 100 angstroms from the position they would occupy if the bridges were accurately perpendicular to the thick filaments. It is clear therefore that, in order to produce the much larger overall sliding movement, some type of repetitive interaction of the cross-bridges with the actin filaments is necessary. One possibility might be that the cross-bridges move to and fro in a cyclical manner, attaching to the actin filaments and pulling them toward the center of the A-band on one part of their stroke, and detaching again prior to their return stroke. Alternatively, the cross-bridges might remain rigidly fixed in position while repetitive internal changes in the actin filaments enabled them to crawl along the series of fixed points so provided. But whatever the details, the basic idea was that the cross-bridges were in direct contact with the actin filaments when force was developed, and that they were the mechanical agents through which the force was transmitted.

Since the probable free energy of the chemical reaction apparently most closely linked to contraction is known (*8*), it can be estimated that the splitting of an amount of adenosine tri-

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phosphate equivalent to one molecule for each myosin cross-bridge throughout the muscle would provide sufficient energy for the actin and myosin filaments in each half-sarcomere to move past each other by 50 to 100 angstroms when the muscle was shortening against a maximal load. This is consistent with (but of course, does not prove) a model in which one molecule of adenosine triphosphate is split at a cross-bridge during one cycle of its action and in which each cross-bridge can go through its tension-generating cycle only once during each 50- to 100-angstrom relative movement of the filaments. These requirements have the obvious corollary that the probability of splitting is low or zero when this cycle is not completed. In this way chemical-energy release in the muscle can be controlled (i) by the tension developed (proportional to the number of bridges which had time to attach at any given shortening velocity) and (ii) by the distance shortened (proportional to the number of cycles of attached bridges). In such a system, energy release could be efficiently matched to the work done, as is known to be the case in muscle (*9*). Furthermore, a considerable number of other properties of striated muscle could be explained on this general basis (*3*, *5*); it was desirable, therefore, to investigate the nature and behavior of the cross-bridges in as much detail as possible.

More Detailed Electron-Microscope Observations

Although the cross-bridges were first seen in sectioned muscle in the electron microscope, their appearance under these conditions is unsatisfactory when more detailed information is required. Their arrangement on the thick filaments does not appear very regular, and little internal detail is visible. [Recent x-ray diffraction observation (*10*) has shown that, although the regular structure of the muscle filaments and of the cross-bridges on them is remarkably well preserved by glutaraldehyde fixation (now the method of choice), a very great deal of the regular order is lost during the subsequent dehydration of the specimens prior to embedding.] However, studies of separated muscle filaments, in which we used the negative staining technique, revealed a number of new structural features. These were described in detail several years ago (*11*), but it will be useful to recall briefly some features that are particularly relevant to the present discussion.

The most straightforward evidence that the cross-bridges represent the heavy-meromyosin end of the myosin molecule came from a comparison of the filamentous aggregates of light meromyosin (formed at physiological ionic strength) with aggregates formed under similar conditions by intact myosin molecules (see Fig. 2). The former were seen to consist of needle-shaped structures many microns long and of various widths, up to several thousand angstrom units. The surfaces of these light meromyosin filaments were perfectly smooth. On the other hand, the filaments formed by the aggregation of whole myosin molecules had large numbers of projections on their surfaces over most of their length. These filaments varied in thickness but were usually less than about 200 angstroms in diameter, and they were usually shorter than the light-meromyosin filaments. Moreover, these synthetic myosin filaments were very similar in appearance to the "natural" thick filaments prepared directly from mechanically disrupted muscle. It was apparent, therefore, that the projections revealed by the negative staining method were equivalent to the cross-bridges in the sectioned material, and that, since they were absent from the light-meromyosin filaments, they must be associated with the heavy-meromyosin part of the molecule. Furthermore, since the cross-bridges seen in sections were of the order of 40 to 50 angstroms wide by 120 angstroms long (and it was known that lateral shrinkage during processing had probably reduced the longer dimension of the cross-bridge by about 20 percent) and since isolated heavy-meromyosin molecules examined by the shadow-casting

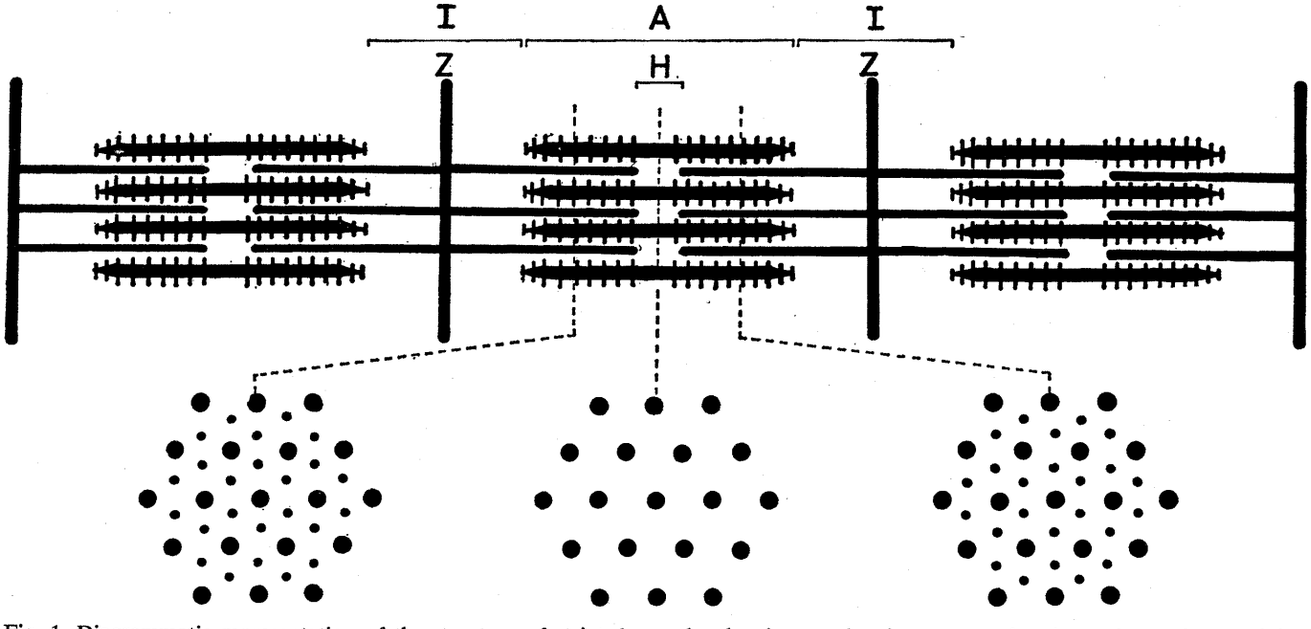


Fig. 1. Diagrammatic representation of the structure of striated muscle, showing overlapping arrays of actin- and myosin-containing filaments, the latter with projecting cross-bridges on them. For convenience of representation, the structure is drawn with considerable longitudinal foreshortening; with filament diameters and side-spacings as shown, the filament lengths should be about five times the lengths shown.

technique (*11*, *12*) showed a globular region of about 40 to 50 by 200 angstroms with a short tail about 400 angstroms long, it was concluded that the cross-bridges represented the globular region, and that the tail must lie approximately parallel to the backbone of the filament. Continuing this line of argument, it seemed reasonable to suppose that the adenosine triphosphatase and actin binding sites would be located in the globular region of the heavy-meromyosin molecule.

Further arguments which need not be repeated here indicated that the myosin molecules were arranged in the thick filaments with a definite structural polarity, so that the heads of the molecules were always directed away from the midpoint of the filaments (see Fig. 3); thus all the cross-bridges in one half of an A-band have the same polarity, and this polarity is reversed in the opposite half of the A-band. If the sets of actin filaments in each half-sarcomere are to be drawn toward the center of the A-band, they must be acted on by sliding forces directed in opposite senses in either half of the A-band. It seems a reasonable arrangement that this directional specificity should be established by the structure of the filaments and be embodied in the orientation of the active sites. It seems likely that these sites would interact in a stereospecific manner with the actin filaments, so that reversing the orientation of the cross-bridges would reverse the direction of the force developed. A corresponding reversal of polarity in the actin filaments would be expected on either side of the Z lines, and this also was found.

These observations therefore reinforced the view that the sliding force was developed as a consequence of direct physical contact between the heavy-meromyosin cross-bridges of the thick filaments and the actin units in the thin filaments.

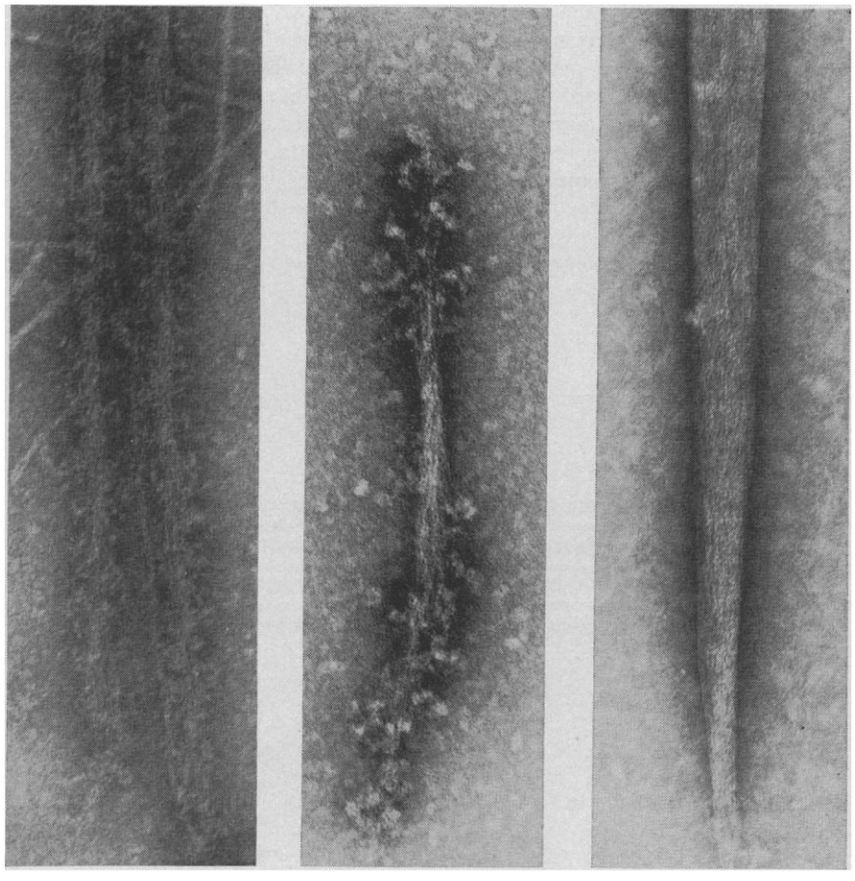


Fig. 2. Electron micrographs of negatively stained preparations of (left) natural thick filaments, prepared directly from homogenized muscle; (middle) synthetic filament formed by aggregation of purified myosin in 0.1*M* KCl; and (right) synthetic filament formed by aggregation of light meromyosin in 0.1*M* KCl. Projecting cross-bridges can be seen on the natural and synthetic filaments in which whole myosin is present —that is, in which both the heavy- and light-meromyosin parts of the myosin molecule are represented. The filaments containing light meromyosin alone have no projections (about × 162,000).

Biochemical Evidence about Actin-Myosin Interaction

An intimate interaction between these molecules during contraction is also indicated by other important lines of evidence, the first of which is concerned with the enzymatic behavior of myosin. Purified myosin, in the presence of concentrations of magnesium and calcium ions similar to those expected in muscle during activity, has relatively low adenosine triphosphatase activity (*13*). However, at the same magnesium and calcium ion concentrations, but in the presence of actin, under conditions where combination between actin and myosin is known to take place in the absence of adenosine triphosphate (that is, at low ionic strength), the adenosine triphosphatase activity is greatly enhanced (about 20-fold or more) (*14*) and approaches that required to account for the known rate of energy release in a muscle (*15*). There is therefore a very strong presumption that the activating influence of actin in the presence of adenosine triphosphate is exerted by a direct physical combination with myosin, even if only a transitory one, for some part of the cycle in which adenosine triphosphate is split. Moreover, a force-generating link between the actin and myosin filaments is required for contraction, and this link has to provide some form of two-way coupling between the performance of mechanical work and the splitting of adenosine triphosphate, so that not only is the energy from the reaction transformed into mechanical work but, *unless* the mechanical work can be performed, the reaction is inhibited. It is very difficult to believe that this link is not provided by actual combination of actin with the heavy-meromyosin cross-bridge.

Recent work by Ebashi and his coworkers (*16*) and by others (*17*, *18*) has added further force to this argument. The work of Annemarie Weber, of Hasselbach, and of Ebashi had shown earlier (*19*, *20*) that the adenosine triphosphatase activity of unpurified actomyosin and of myofibrils can be regulated in vitro by the concentration of calcium ions, a change in concentration from $10^{-7}M$ to $10^{-5}M$ being adequate to increase the activity 20-fold or more, from that characteristic of myosin alone to the full activity of actin-activated myosin. There is good evidence (*21*) that an analogous process occurs in vivo and that release of cal-

cium from the sarcoplasmic reticulum and its subsequent rebinding there when the muscle relaxes permits contraction to be controlled by external electrical signals conducted inward from the sarcolemma along the so-called T-system of the reticulum. However, this left unspecified the exact site of action of the calcium.

It was noticed some years ago (*17*) that the adenosine triphosphatase activity of extensively purified actomyosin was insensitive to the absence of calcium; unlike the activity in unpurified systems, it continued high when calcium was withdrawn. The significance of this was not clear at first, for the effect might have been due to some slight change in the properties of the myosin molecule itself. However, the situation was dramatically clarified when Ebashi (*16*) showed that calcium sensitivity could be restored to such systems by adding back a certain protein fraction. This fraction was shown subsequently to contain two principal protein components—tropomyosin B (*22*) and a new protein, troponin (*23*). Ebashi and his co-workers have shown (*24*) in a very ingenious way that the calcium seems to act on the troponin moiety rather than directly on the actomyosin. Moreover, although purified actomyosin can bind about 1 mole of calcium per mole of myosin, this calcium is not in itself adequate to cause activation of the adenosine triphosphatase in the presence of the troponin-tropomyosin system, and additional calcium has to be provided (*20*), presumably to combine with the troponin.

There has been a good deal of evidence for several years (*5*, *25*) that tropomyosin is present in the thin filaments, as well as actin. This has been confirmed by fluorescent antibody studies by Pepe (*26*) and by Endo and others (*27*), who have also demonstrated the presence of troponin in the same part of the sarcomere—that is, the region occupied by the thin filaments. Ebashi and his co-workers have also shown biochemically that troponin combines with the tropomyosin-actin complex (*23*) but not with myosin (*28*).

Thus, there is very compelling evidence (i) that troponin functions as a safety catch, preventing activation of myosin adenosine triphosphatase by actin when calcium is absent, but allowing the activation to occur as soon as calcium can be bound by the troponin (in other words, troponin appears to act as an allosteric regulatory subunit), and (ii) that troponin is structurally part of the thin filaments. Once again, it is very difficult to believe that this highly effective and sophisticated control system does not depend on a direct physical interaction between the actin filaments and the myosin cross-bridge, and on some form of interference by troponin with this interaction unless calcium is present.

Fig. 3. Diagrammatic representation of the mode of aggregation of myosin molecules to form filaments whose structural polarity reverses at the midpoint. The light-meromyosin parts of the myosin molecules form the backbone of the filaments, and the globular ends of the heavy-meromyosin components form the projecting cross-bridges. Since these will be oriented in opposite senses in the two halves of the A-bands, they could generate sliding forces which are always directed toward the center of the bands.

Problem of Variable Filament Separation

Further information about the way the cross-bridges are involved in contraction is given by the relationship between isometric tension and sarcomere length (*29*), and by the correlation of these observations with the lengths of the actin and myosin filaments (*30*) and the way in which the cross-bridges are distributed (*11*). The active tension generated by a muscle at different lengths (greater than rest length) is very accurately linearly proportional to the number of cross-bridges overlapped by the actin, decreasing to zero when the muscle is stretched to the point where overlap just ceases. This strongly suggests that each cross-bridge develops a given amount of tension whatever the extent of overlap between the filaments, and that the number of bridges attached at any one time at a given muscle length is proportional to the number of bridges overlapped by the actin filaments. It seems much less likely that the linear form would arise accidentally, from a coincidental variation of the tension per cross-bridge, and of the probability of attachment, with sarcomere length, which happened to give a constant product at all sarcomere lengths.

This behavior may at first seem very straightforward, and easily accounted for by supposing that a cross-bridge undergoes some unique set of structural changes when it interacts with actin and splits adenosine triphosphate and that these changes enable it to develop a fixed amount of tension. However, the first signs of a real difficulty with this simple mechanical picture appear when we take into consideration measurements of the side spacing between the actin and myosin filaments at different muscle lengths. It was found some years ago (*31*), and later confirmed (*32*), that the filament lattice in a live muscle exhibits the same constant-volume behavior as the whole muscle itself; the filaments move closer together as the muscle is stretched and move further apart when it is allowed to return toward rest length, their separation varying inversely as the square root of the muscle length. Thus, between sarcomere lengths of 2.8 and 2.0 microns (equilibrium length), the side spacing will increase by about 18 percent of its original value. If the center-to-center separation of the actin

and myosin filaments has a value of about 210 angstroms at a sarcomere length of 2.8 microns, the increase in distance will be about 40 angstroms. This is a very large distance indeed when one is thinking in terms of the kinds of interaction between protein molecules or protein subunits necessary to produce the highly specific conformational changes associated with the regulation of enzyme activity, which is one of the outstanding features of the system we are considering. If close contacts are to be preserved between the cross-bridge and the actin filament, then the cross-bridges must in some way be able to adapt themselves to these changes in spacing between the filaments and yet must still function in precisely the same way.

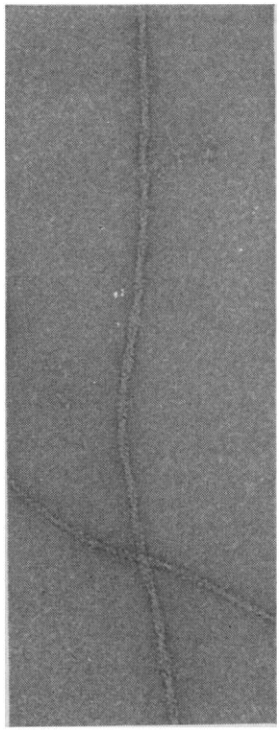
Assumed changes in orientation of the globular region which would enable it to adopt a more perpendicular orientation and bridge the larger gap at the shorter sarcomere length do not really provide a satisfactory solution to this problem, since the change in angle would be so great; a cross-bridge 160 angstroms long would have to alter its initial tilt by about 41 degrees. If a specific set of structural changes occur at the cross-bridges during adenosine triphosphate splitting and tension development, it is difficult to imagine how the cross-bridges could develop the same longitudinal component of tension over such a wide range of orientations. Nevertheless, such constancy in behavior is strongly indicated by the linear form of the length-tension diagram.

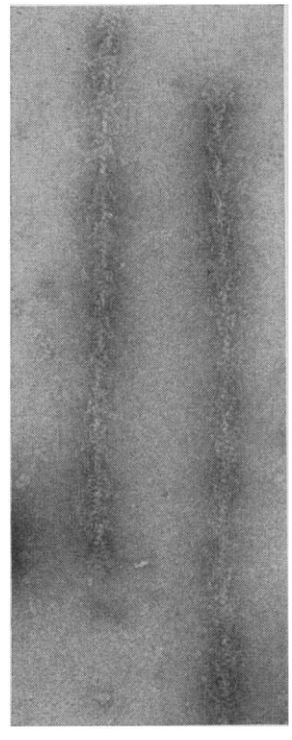
At one time a conceivable way out of this difficulty was to suppose that the interfilament spacing, although variable in resting muscle, always adjusted itself to a constant value during contraction. However, this has been shown not to be the case by Elliott, Lowy, and Millman (*33*), and almost the same range of interfilamentous spacings is exhibited by an actively contracting muscle as by a muscle at rest. Indeed one can begin to see why such variations in spacing should be inherent in the system. A fast and efficient muscle should always operate with a low coefficient of internal friction, whether it is actively shortening or being passively stretched. It appears that this is achieved in nature by sliding of the filaments past each other on a cushion of long-range electrostatic forces of the kind envisaged by Rome (*34*) and by Elliott (*35*). In such a system, changes in the extent of overlap of the filaments seem bound to alter this force balance (*35*) and hence to change the equilibrium separation of the filaments. Thus, satisfactory operational characteristics for a muscle may not be compatible with a fixed side spacing between filaments.

Nevertheless, a considerable body of strong evidence does indicate, as we have seen, that physical contact between the cross-bridges and the actin filaments must take place during contraction; thus a very real and interesting difficulty does exist here, and I will now discuss some recent structural evidence which may provide clues as to how this paradox can be resolved.

Subunit Order and Negative Staining

Electron-microscope observations on separated muscle filaments, made by means of the negative staining technique, show significant differences in





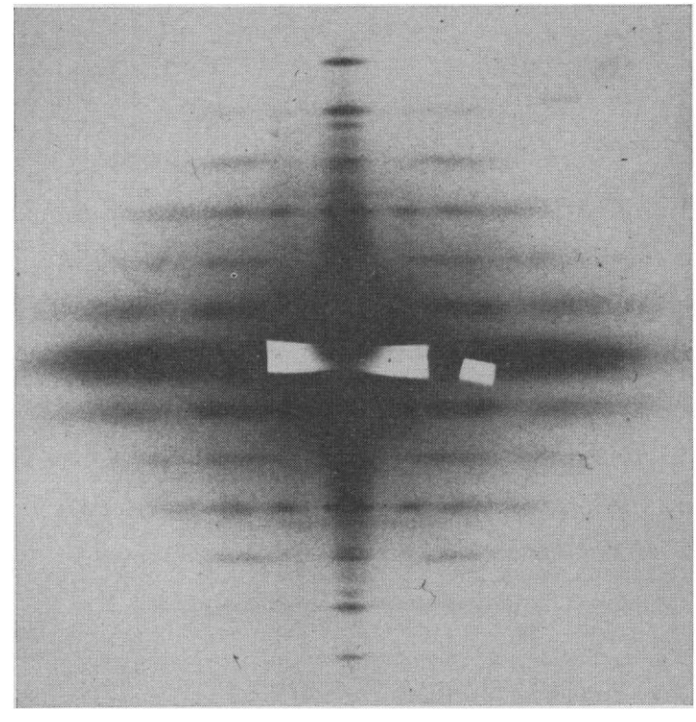


Fig. 4 (left). Electron micrograph of negatively stained actin filament, showing the double helical arrangement of two chains of globular subunits twisted around each other. The subunit repeat in each chain is about 55 angstroms, and the cross-over points of the two chains are 360 to 370 angstroms apart. Fig. 5 (center). Electron micrograph of negatively stained actin filament "decorated" with heavy meromyosin. The polarity of the structure is shown by the "arrowhead" appearance, and it is evident that a quite regularly ordered arrangement of the heavy-meromyosin units is preserved. (Compare the arrangement of cross-bridges in Fig. 2, left and middle) (about × 155,000). Fig. 6 (right). Low-angle x-ray diffraction pattern from living frog sartorius muscle (fiber axis vertical). The reflections form a system of horizontal layer lines (with a repeat of ~429 angstroms) which arise from the helical arrangement of cross-bridges on the thick filaments.

the regularity of the visible subunit repeats (*11*). The actin filaments show better structural preservation in this respect than the myosin filaments do. In the former, the double-helical arrangement of G-actin units can be seen quite well (Fig. 4), whereas virtually no trace of an ordered helical structure can be seen in the arrangement of the projections on the myosin filaments (Fig. 2). Since it was believed on general grounds [and was demonstrated by x-ray diffraction (*36*)] that the cross-bridges are arranged in a regular fashion in the intact muscle, it was apparent that this regular arrangement must have been greatly disturbed during the negative staining process. In view of the known lability of myosin, this was not altogether surprising, but it was somewhat unexpected to find that myosin or heavy meromyosin complexed to actin gave a compound filament showing a considerable amount of structural regularity in the arrangement of the subunits bound to the outside of the actin filament (Fig. 5). Though the significance of the differing amounts of order in the two situations (that is, cross-bridges on the outside of myosin filaments and isolated cross-bridges attached to actin filaments) is not easy to assess, it is apparent that the attachment to actin must in *some* sense be a much more rigid one than that to the backbone of the myosin filaments.

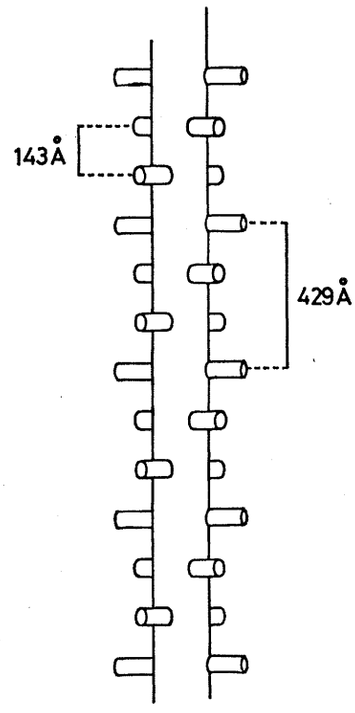


Fig. 7. Diagram of cross-bridge arrangement on thick myosin-containing filaments of frog sartorius muscle, which would account for the observed x-ray pattern.

New X-ray Diffraction Results

Further evidence about the structure and the character of the filaments has come from detailed studies on the low-angle x-ray diffraction patterns from muscle under differing conditions (*37*). These observations have been described at length elsewhere; here I will mention briefly those findings which bear closely on the present problem. The results show that in live striated muscle of vertebrates the projections on the thick filaments are arranged on a 6/2 helix. At a given level, two bridges project out directly opposite each other on either side of the backbone of the thick filament. The next two bridges occur 143 angstroms further along the filaments and are rotated relative to the first pair by 120 degrees. This arrangement continues, so that the structure as a whole repeats at intervals of 3 × 143, or 429 angstroms (see Figs. 6 and 7).

The distribution of x-ray intensity along the layer lines can be accounted for very satisfactorily on the basis of a model in which projections are attached to the backbone of the thick filaments at a radius of about 60 angstroms from the center of the thick filament and extend outward to a total radius of about 130 angstroms. Now the distance to the surface of the actin filaments from the center of the myosin filaments is about 190 angstroms in a muscle at rest length, and bridges extending out to this distance would give a predicted x-ray intensity appreciably different from the observed one. Of course, the result might merely indicate that the effect of disorder on the cross-bridges was more marked at larger radii, but the simpler explanation would be that the cross-bridges in a resting muscle do not extend all the way out to the actin filaments.

A most significant feature of the cross-bridge pattern is the fact that, although the pattern is strongly developed at low angles (at spacings greater than about 50 angstroms), the reflections, especially the off-meridional ones, fade out very quickly at higher angles. This shows that on any given thick filament there must be a considerable amount of disorder in the helical arrangement of the bridges. The regularity of the arrangement can be contrasted, for example, with the highly ordered arrangement of protein subunits in the rods of tobacco mosaic virus, oriented gels of which will give detailed x-ray patterns out to spacings of only a few angstrom units. It is clear that the bridges in a resting muscle are not at all precisely fixed in position on the thick filaments. Again, this is a surprising result, for one might have expected that structures involved in the precise and intricate mechanochemical interactions of contraction would need to be positioned in a very precise and rigid fashion.

The x-ray reflections from the actin filaments show that the G-actin units are arranged on a nonintegral helix with subunits repeating at intervals of 54.6 angstroms along either of two chains which are staggered relative to each other by half a subunit period (27.3 angstroms), and which twist around each other with cross-over points 360 to 370 angstroms apart, so that the pitch of the helix formed by either of the two chains is 720 to 740 angstroms (see Fig. 8). Again, the actin reflections show something of the same disorder that characterizes the reflections from the myosin filaments; in this case, prominent meridional reflections out to 6 angstroms or less show good ordering in a purely axial sense, but off-meridional reflections at higher angles are extremely weak, indicating relatively poor helical ordering of the subunits. This suggests that the helices may be able to twist and untwist to some extent, but that the axial repeat of the subunits remains rather constant.

When a muscle loses adenosine triphosphate and goes into rigor, it becomes rigid and inextensible, a phenomenon which has been interpreted in terms of the attachment of a large number, if not all, of the cross-bridges to the actin filaments. Since neither the subunit repeats nor the helical repeats of the myosin and actin filaments are the same—indeed, a near match of one cross-bridge with an actin monomer oriented in the right direction occurs only once every several thousand angstrom units along the filaments—such an attachment can take place only if some part of the structure alters its configuration from that in the resting state. When muscles in rigor were examined, it was found that the low-angle x-ray pattern from the cross-bridges changes very considerably, while the part of the pattern arising from the actin filaments remains almost constant. The entire system of myosin layer lines based on the 429 angstrom

helical period disappears, showing that a high proportion of the whole mass of each of the cross-bridges moves away from the position it occupied in the resting muscle. The layer-line pattern is replaced by a somewhat more disordered but nevertheless quite strong system of layer lines now based on a helix of pitch about 720 angstroms—approximately the pitch of the chains of monomers in the actin filaments. This indicates that a high proportion of the cross-bridges have attached in a systematic way to the subunits in the actin filaments.

The most surprising feature of the new pattern, however, is the continued presence of a strong meridional reflection at a spacing almost unchanged in value from that in resting muscle at 143 angstroms. This reflection arises from the meridional repeat of the cross-bridges, and its continued presence shows that, although the helical features of the arrangement of bridges has changed so as to enable them to match up more easily with actin monomers—that is, although they have moved in an azimuthal (and possibly a radial) direction, so as to lie on a helix of different pitch—this has been accomplished with very little movement in an axial direction. We can see that five subunit periods with an unchanged repeat of 143 angstroms would fit quite closely with the new pitch of about 720 angstroms and that, with this repeat, a considerable number of near matches could be made with subunits on the actin filaments (*37*). Thus, it seems that the cross-bridges are able to swing bodily around the thick filaments, keeping their axial positions approximately constant but changing their azimuth, and probably their radius too.

A similar type of behavior is observed in actively contracting muscles, in which the off-meridional layer-line pattern becomes very much weaker, although here the "resting" pattern is not replaced by a new one—a fact which indicates that the bridges do not settle down to some new regularly ordered arrangement. Once again, the strong 143-angstrom meridional reflection decreases in intensity to a much smaller extent than the off-meridional reflection and changes its spacing by only about 1 percent from the value characteristic of resting muscle. Again, the actin pattern appears to be unchanged in the actively contracting muscle.

The constancy of meridional repeating distances fitted in very well with the earlier observations of constancy of filament length during contraction, but the behavior of the cross-bridges, as in the case of rigor, was at first very unexpected and puzzling. How could the bridges move to a new helical arrangement yet leave undisturbed the bonding pattern which gave rise to their meridional repeat?

Possible Solution to the Paradox

This puzzle began to resolve itself as the concept of cross-bridges attached at their bases to the backbone of the filaments but able to tilt was abandoned in favor of a model having a rather different construction (*37*). This was given support by Lowey's timely characterization (*38*) of a helical subfragment, derived from heavy meromyosin and *soluble* at physiological ionic strength; this subfragment does not aggregate under these conditions either with itself or with light meromyosin. Now, heavy meromyosin itself is soluble at physiological ionic strength and is known to possess a globular head, and a short linear tail similar in appearance to the rest of the myosin molecule—that is, to the light-meromyosin subunit, which forms aggregates at physiological ionic strength. Previously it had seemed possible that the linear portion of heavy meromyosin might aggregate too, if it were separated from the head part of the molecule. However, this turned out not to be the case, and it therefore became apparent (*37*, *39*) that the globular part of myosin—the part forming the visible cross-bridge—could be attached to the backbone of the thick filaments by a linear region of the molecule (about 400 angstroms long), which would not be bonded along its length to the surface of the thick filaments but would be attached only at one end, at the junction to the light-meromyosin part of the molecule.

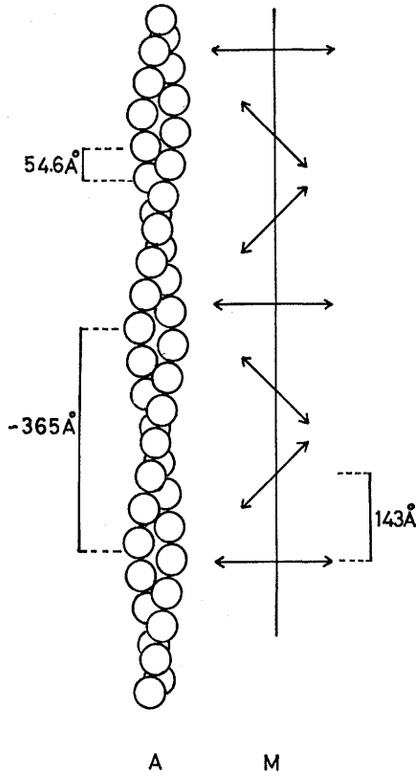


Fig. 8. Diagram of the arrangement of G-actin monomers in actin (*A*) filaments, derived from x-ray diffraction and electron-microscope observations. Both the pitch of the helix and the subunit repeat differ from those of the myosin (*M*) filaments, indicated schematically alongside. Thus, cross-bridges between filaments would act asynchronously, and a sequence of them would develop a fairly steady force as the filaments moved.

The possibility thus began to emerge that this link might provide the movable attachment which was being called for by the results of the x-ray experiments. Since the junction between heavy and light meromyosin is susceptible to tryptic digestion, it might represent a less perfectly α-helical region of the molecule and hence a region of greater flexibility; similarly, the junction between the linear part of heavy meromyosin and the globular part of the molecule is also susceptible to enzymatic digestion (by papain, or by more prolonged tryptic action), and so it too might provide a flexible coupling. Thus, with two flexible joints, the orientation of the "head" of the molecule could be maintained when the link swung further out from the backbone of the filaments (Fig. 9).

The great advantage of this model is that it permits direct myosin-actin interaction to take place over a wide range of interfilament spacing, as illustrated in Fig. 10. It may be seen that the cross-bridges can be attached at the same orientation to the actin subunits over a considerable range of filament spacings. Thus all the difficulties discussed above are circumvented.

Models having some analogies to this one but not concerned with the two major structural components of heavy meromyosin have been suggested by Pepe on the basis of his antibody-

staining experiments (*40*), but I have reservations about some of the arguments involved. Hanson (*41*) has also recently reviewed the possibilities.

This model of course makes it possible to account for the meridional x-ray data, which show, essentially, that the globular part of the heavy-meromyosin molecules can move circumferentially around the long axis of the thick filaments to some new position yet still be held with approximately the same axial repeat. The axial repeat of the bridges is fixed by the packing of the light-meromyosin part of the molecules in the backbone of the thick filaments, which remains constant. If the end of the cross-bridge is constrained to move to a new position by attachment to actin, then, instead of requiring a major change in orientation of the globular region, this model will allow the whole globular region to move circumferentially to a new position, keeping its orientation approximately constant, so as to match up with a site at the appropriate level on one of the actin filaments nearby. The spacing of the 143-angstrom meridional reflection could be maintained and its intensity would still be quite strong, yet virtually all traces of the original 429-angstrom helical reflections would be lost.

Furthermore, the flexibility of the attachment of the cross-bridges to the backbone also offers a possible explanation for the disordered appearance of the x-ray reflections at higher angles and for the difficulty of preserving an ordered arrangement in material processed for examination in the electron microscope. Additionally, the model has the advantage of providing a plausible role for the various structural parts of the myosin molecule, especially the "soluble" linear portion of heavy meromyosin.

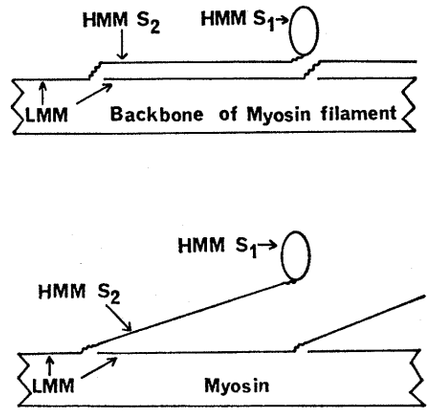


Fig. 9. Suggested behavior of myosin molecules in the thick filaments. The light-meromyosin (LMM) part of the molecule is bonded into the backbone of the filament, while the linear portion of the heavy-meromyosin (HMM) component can tilt further out from the filament (by bending at the HMM–LMM junction), allowing the globular part of HMM (that is, the S_1 fragment) to attach to actin over a range of different side-spacings, while maintaining the same orientation.

Additional X-ray Evidence

It seemed worth while, therefore, to consider this model seriously and to look for additional evidence concerning its validity. For this reason, a further study was made of the equatorial x-ray reflections from resting muscles and from muscles in rigor. These reflections would be expected to show up any changes in the average radial density distribution in a direction at right angles to the long axis of the filaments which might take place when the muscle went into rigor, and they could therefore make it possible to detect changes in the radial positions of the cross-bridges. These changes would also show up in the axial reflections, and indeed strong indications that they were present had already been detected (*37*). However, the effects are more difficult to interpret in this case because of the disorder present in the helical structure.

It may be recalled that striking differences had already been noticed some years earlier (*31*) in the relative intensities of the equatorial reflections from muscle, when patterns from live relaxed specimens were compared with patterns from muscle in rigor or

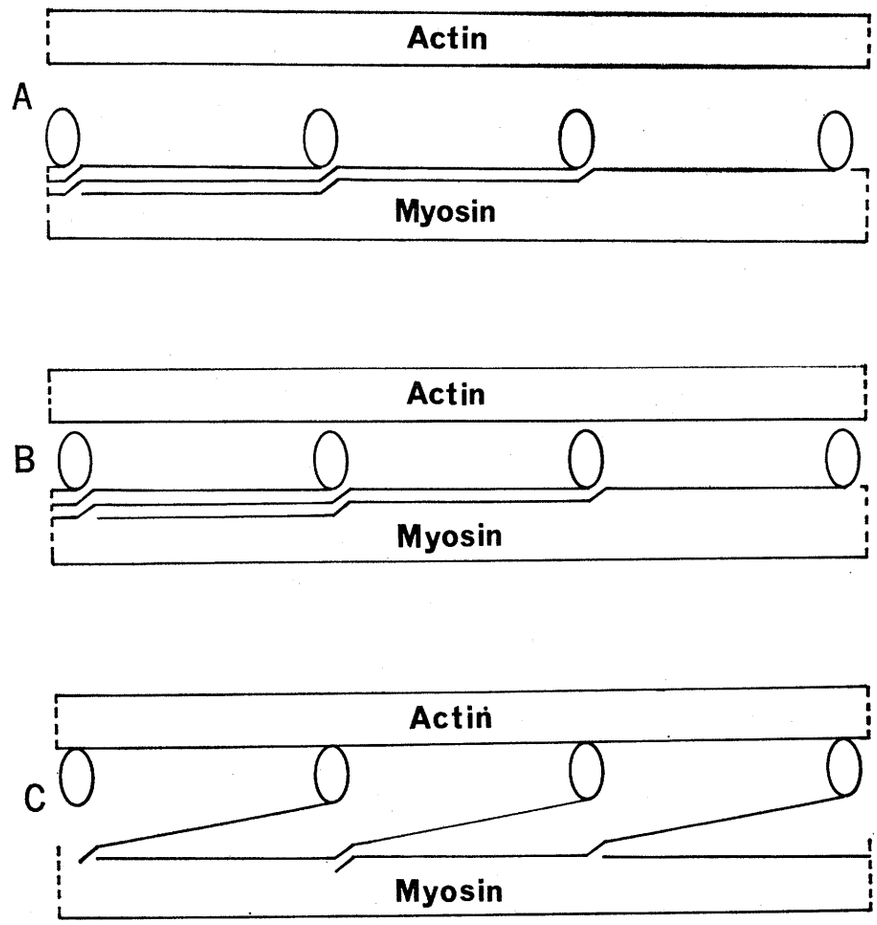


Fig. 10. Diagram showing relative positions of filaments and cross-bridges at two different interfilament spacings [(A) 250 angstroms and (B) 200 angstroms] corresponding, in frog sartorius muscle, to sarcomere lengths of ~2.0 and ~3.1 microns. The x-ray diagram (not shown) suggests that in a relaxed muscle the cross-bridges do not project very far toward the actin filaments. During contraction, or in rigor, the cross-bridges could attach to the actin filaments by bending at two flexible junctions, as shown in (C).

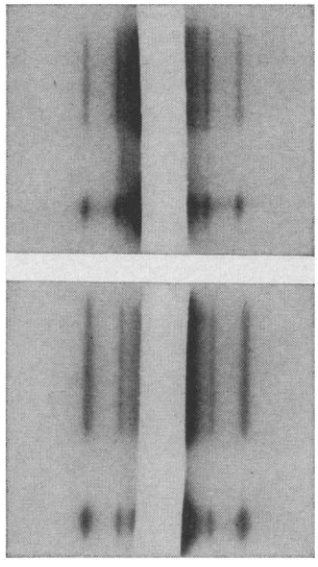


Fig. 11. Low-angle equatorial x-ray patterns from rabbit psoas muscle: (top) live; (bottom) in rigor. The patterns show the 10 and 11 reflections from the hexagonal lattice of myosin and actin filaments. The reversal of the relative intensities of the reflections is believed to be caused by the cross-bridges reaching farther out from the myosin filaments in rigor and attaching to the actin filaments at the trigonal positions.

after glycerol extraction. Subsequently, doubts were expressed (*32*) about the validity of these findings, but when this question was reinvestigated recently (*42*) it was demonstrated that there are indeed large differences in the relative intensities of the 10 and 11 reflections when patterns from live muscle and from muscle in rigor are compared at the same sarcomere length (Fig. 11). Quantitatively, the changes indicate that an amount of material equal to about 30 percent of the total original mass of the thick filaments is transferred to the vicinity of the thin filaments, at the trigonal positions, when a muscle passes into rigor. This transfer could be accounted for very well if the globular ends of the heavy-meromyosin molecules, originally extending only partway out from the backbone of the thick filaments, reached farther out when the muscle was in rigor, and attached to the surface of the actin filaments. Further support for this interpretation is given by electron-microscope observations of cross sections of muscle. Not only is the change in the relative amount of material associated with the thick and thin filaments very evident in such cross sections when the muscle passes into rigor but, furthermore, a readily visible *reversal* of the change can be produced by treating (before fixation) a muscle in rigor with a "relaxing" solution containing adenosine triphosphate and ethylenedinitrilotetraacetic acid, a procedure which would be expected to detach the cross-bridges again from the thin filaments.

Thus a number of different structural observations all strongly support the idea that the active part of the myosin molecule—namely, the globular-head region containing the adenosine triphosphatase and actin binding sites—is attached to the backbone of the thick filament by means of two separate flexible couplings with a linear region in between them, so that the "heads" on the thick filaments can attach themselves to the actin filament over a considerable range of different actin-myosin spacings, and yet always preserve exactly the same orientation relative to the actin. Let us examine some of the consequences of this possible model.

Site of the Structural Change

Since it is one of the postulates of the model that the junction between light and heavy meromyosin is flexible, and since we are supposing that the linear part of heavy meromyosin lies approximately parallel to the axis of the filaments, it is clear that active bending of this particular junction is not a likely source of the longitudinal contractile force. The linear portion of heavy meromyosin does not seem likely to be the seat of the contractile machinery either, since the actin-activated adenosine triphosphatase activity of the head region can function normally after it has been removed (*43*), and since it is not easy to imagine that relatively distant changes in the head region could cause this two-chain α-helical structure to fold up to a shorter length. The junction between the linear part of heavy meromyosin and the globular region also seems an unfavorable position for the force-generating mechanism, for two reasons. First, if the globular part of heavy meromyosin attaches at a constant angle to actin, then the angle formed between the linear and the globular parts will vary somewhat with filament spacing (and indeed it is for this very reason that we have supposed the junction to be a flexible one); thus the mechanism would have to be capable of acting over a variable range of configurations. Second, such a mechanism would require that the linear part of heavy meromyosin be a *completely* rigid rod, since it would have to sustain the same couple that was being developed at the junctional region.

Thus, it seems unlikely that any of the structural elements through which the globular parts of cross-bridges are attached to the backbone of the thick filaments could provide the structural requirements necessary for the *development* of a longitudinal sliding force, though these attachments could perfectly well sustain a force developed elsewhere. Indeed, the orientation of the myosin molecules in the filaments is such that the linear part of heavy meromyosin would always be under tension during contraction, a form of stress which this type of structure seems well adapted to sustain.

Clearly, the most likely seat of the force-developing mechanism is the globular part of heavy meromyosin and its attachment to the actin filaments. We have already seen, from the ordered structures visible in negatively stained preparations of actin "decorated" with heavy meromyosin, that this attachment seems to be a rather rigid one. It is therefore perhaps more profitable to reverse one's usual picture of the structure and to think of the cross-bridges (for part of their cycle, anyway) as being based on the *actin* filaments, and as being attached to the myosin filament by a link which could be as flexible as a piece of thread, provided it was inextensible. Changes in orientation of the cross-bridge relative to the actin filament to which it is attached will then give rise to a relative sliding force between the filaments in the manner required.

Such a change of orientation could be brought about in several ways. The two globular units which make up the head of heavy meromyosin could function essentially independently, and each could undergo either a change in shape or a change in the angle at which it attached to the actin filaments (Fig. 12A). Alternatively, the mechanism might depend specifically on the dual structure in the head region. Perhaps one subunit could attach with the head perpendicular to the actin filament, while the second subunit would attach only with the head bent at an angle to the actin fila-

ment. Another possibility is that, in the intact molecule, interactions occur between the two head subunits, and that during the splitting of adenosine triphosphate the relative positions of the subunits change. This could alter the profile of the surface which is applied to the actin filament and hence could alter the angle of attachment (Fig. 12B).

No doubt other schemes could be devised, all having in common the basic feature that they depend on an active change in the effective angle of attachment of the globular part of the cross-bridge to the actin filament during the active stroke, rather than on a change in orientation of the cross-bridge which could, in principle, fully manifest itself in the absence of actin. (Given such a scheme, it would not be surprising to find that the configurational changes that occur in purified myosin during the splitting of adenosine triphosphate are relatively minor ones). And while this view has arisen from consideration of the x-ray diffraction observations on vertebrate striated muscle, it derives additional support for some of its features (especially the tilt *direction*) from the elegant observations of Reedy, Holmes, and Tregear (*44*) on the angling of the cross-bridges when they attach, in rigor, to the actin filaments in insect flight muscle. Both Reedy and his associates and Pringle (*45*) have suggested that such "angling" also occurs during the oscillatory contraction of this muscle.

Thus, interest is focusing now on the mode of attachment of the cross-bridge to the actin filament, and there are obvious ways of exploring this in greater detail. However, it is becoming increasingly clear that a full understanding of this or indeed of any other molecular mechanism is likely to require that we solve the full three-dimensional structure of the molecules concerned down to atomic resolution by crystallographic techniques. It has always seemed very unlikely that intact myosin itself could be assembled into crystalline arrays of the requisite degree of regularity, but the isolation in a fairly pure form (*46*) of a globular subunit still possessing many of the relevant properties of myosin has at last brought a real possibility of solving the problem in detail, provided this protein subunit can be crystallized.

Summary

To summarize, then: the contraction of striated muscle is brought about by some mechanism which generates a relative sliding force between the partly overlapping arrays of actin and myosin filaments. There is very strong evidence that cross-bridges projecting out from the myosin filaments, and carrying the adenosine triphosphatase and actin binding sites, are involved in the generation of this force in some cyclical process. However, it appears that the mechanism must satisfy two conflicting requirements: (i) that the force be produced as a result of a precisely determined set of structural changes in a protein complex consisting of actin, myosin, and other components, and be associated with the splitting of a molecule of adenosine triphosphate; (ii) that the force-generating mechanism can work equally well over a considerable range of side spacings between the actin and myosin filaments. Recent evidence suggests that these requirements may be satisfied in the following way: the actual force-generating structure is attached to the backbone of the myosin filaments by a linkage, 400 angstroms long, which has flexible couplings at either end; the force-generating structure can therefore attach itself to the actin filament, in a constant configuration, and undergo exactly the same structural changes and produce the same longitudinal force over a wide range of interfilament separations. The muscle structure is arranged so that the linkage is under tension, not compression, when a contractile force is being generated, and so the force can be transmitted without difficulty. It is suggested that the characteristic feature of

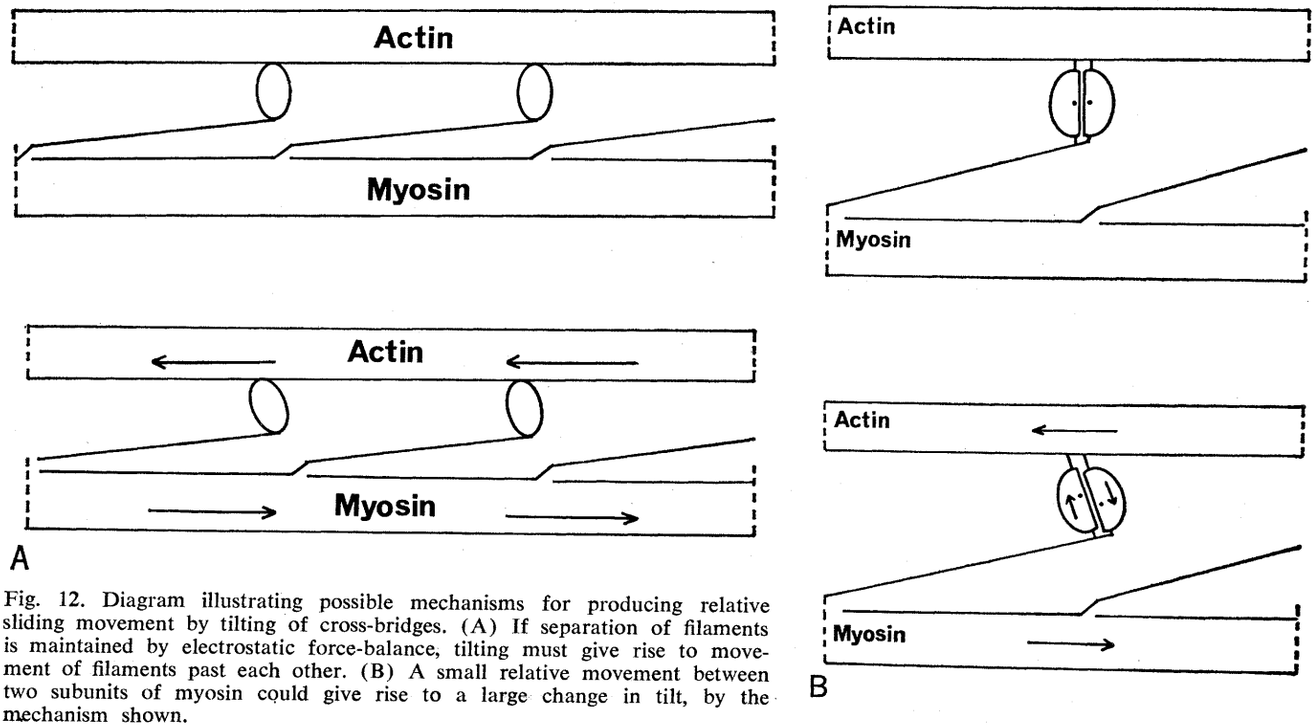


Fig. 12. Diagram illustrating possible mechanisms for producing relative sliding movement by tilting of cross-bridges. (A) If separation of filaments is maintained by electrostatic force-balance, tilting must give rise to movement of filaments past each other. (B) A small relative movement between two subunits of myosin could give rise to a large change in tilt, by the mechanism shown.

the contraction mechanism may be a rigid attachment of the globular head of the myosin molecule to the actin filament and an active change in the angle of attachment associated with the splitting of adenosine triphosphate. The availability of purified preparations of "head" subunits now opens up the problem to detailed attack.

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Evolutionary History of the Elephant

A tentative phylogeny of Elephantidae based on morphological and quantitative analysis is given.

Emiliano Aguirre

Elephants, which are among the most popular and decorative of animals, stand as a witness of prehistory, having been a part of the environment of our ancestors. The dinosaur was not contemporary with early man, as many films and stories insist, but the mammoth was. Although prehistoric or extinct elephants are frequently referred to as mammoths, such a designation is not always correct. The true mammoth is but one of many species of extinct elephants; furthermore, it belongs to one of a few genera, which include four or five species that have affinities with the woolly elephant. These different genera and species are grouped by zoologists into a family, Elephantidae. Because this family originated by the beginning of the Pleistocene period, elephants can be considered contemporary with man.

Anthropologists and prehistorians have often attempted to establish a chronology of sites of fossil man through correlations based upon the species of elephant associated with them (*1*), but the systematics of the Elephantidae is quite confused. The documented monograph of Osborn (*2*) established 10 genera and some 59 species of elephants; to these Garutt (*3*) added two more genera. However, many taxonomists have recognized only one genus and no more than five or six valid species. In the museum collections from most major sites there are many samples with dubious identifications and many intermediate forms labeled either with two names or with a composite or new name. It has been assumed that many different species have lived contemporaneously in a single area, as was the case for the sample excavated in the railway trench of San Paolo, Italy, in the first years of this century. Explanations of the phylogeny of elephants have had one feature in common: the patterns for the phyletic trees have agreed with the fashionable evolutionary theories

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